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AOGS REVIEW ARTICLE

Nonsteroidal anti-inflammatory drugs and risk of ovarian cancer: systematic review and meta-analysis of observational studies

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Key words
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Conflict of interest
The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Abstract

Objective. Several observational studies have investigated the association between nonsteroidal anti-inflammatory drug (NSAID) use and ovarian cancer risk, but with conflicting results. We performed a systematic review and meta-analysis of the association between NSAID use and ovarian cancer risk. *Design.* Systematic review and meta-analysis of observational studies published until September 2012. *Setting.* Studies were identified from the PubMed database. *Population.* Fourteen case-control and seven cohort studies were included. *Methods.* Pooled relative risks (RRs) with corresponding 95% confidence intervals (CI) for aspirin and non-aspirin NSAIDs, separately, were calculated. Both fixed and random effect models were applied, but only random effect pooled RRs are presented. Risk estimates for invasive and borderline ovarian tumors combined and for invasive ovarian tumors only were calculated. Furthermore, heterogeneity and publication bias were evaluated. *Main outcome measures.* Ovarian cancer. *Results.* In the combined analysis, a slight inverse association between use of aspirin (RR 0.93; 95% CI 0.84–1.02) and non-aspirin NSAIDs (RR 0.94; 95% CI 0.84–1.06) and ovarian cancer risk was found, although it was not statistically significant. However, the risk of invasive ovarian cancer was significantly reduced with use of aspirin (RR 0.88; 95% CI 0.79–0.98). A similar tendency was observed for non-aspirin NSAIDs, but the results were not significant. *Conclusions.* This meta-analysis showed a slight inverse association between NSAIDs and risk of ovarian cancer. However, data suggest that a chemopreventive effect of NSAIDs may be restricted to invasive ovarian tumors. Further research on NSAIDs and ovarian cancer is needed before definite conclusions can be drawn.

Abbreviations: CI, confidence interval; COX, cyclooxygenase; NSAID, nonsteroidal anti-inflammatory drug; OTC, over-the-counter; RR, relative risk.

Introduction

Ovarian cancer is the most lethal gynecologic malignancy, with an overall five-year survival of approximately 40% (1). Worldwide, about 225 000 women are diagnosed with ovarian cancer each year and 140 000 die from the disease (2). Ovarian cancer is particularly frequent in

Key Message

A chemopreventive effect of NSAIDs may be restricted to invasive ovarian tumors, which emphasizes the importance of analyzing invasive and borderline ovarian tumors separately. Further research on the association between NSAIDs and ovarian cancer risk is warranted.

northern Europe, with age-standardized incidence and mortality rates of 11.8 and 6.5 per 100 000, respectively (3). The high mortality/incidence ratio is caused by the typical late diagnosis of ovarian cancer. In Denmark, fewer than 20% of women have localized tumors at the time of diagnosis (4). Thus, due to the poor prognosis and the challenges with early diagnosis, identification of potential factors for prevention of ovarian cancer has great clinical and public health implications.

There is a solid body of evidence of an association between chronic inflammation and cancer, which forms the basis for the hypothesis of a chemopreventive effect of anti-inflammatory drugs (5,6). The main mechanism of action of nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, is inhibition of the cyclooxygenase (COX) enzymes, which catalyze the synthesis of prostaglandins that play an important role in inflammatory processes. However, NSAIDs have several other pharmacodynamic effects and other chemopreventive mechanisms may exist (5,7). The strongest evidence of a chemopreventive effect associated with use of aspirin and other NSAIDs pertains to colorectal cancer, whereas the evidence of a protective effect of these drugs for other cancer sites is less consistent (8). However, a recent review concluded that the chemopreventive effect of aspirin in colorectal cancer may be extended to several cancers, including ovarian cancer (9). For ovarian cancer, inflammatory conditions such as exposure to talc and asbestos, endometriosis and pelvic inflammatory disease have been implicated as risk factors (10). Furthermore, repeated cell damage and inflammation following ovulation is known to play an important role in the pathogenesis (11,12). These observations together with preclinical studies suggesting an important role for COX in ovarian carcinogenesis (13,14) support the hypothesis of a reduction in risk of ovarian cancer associated with use of aspirin and other NSAIDs.

A number of observational studies have investigated the association between NSAID use and risk of ovarian cancer, and the association has been summarized in review articles of NSAID use and cancer risk (9,15–20). Overall, pooled estimates have been compatible with no effect or a slight inverse association. Among the previous meta-analyses, three have focused specifically on ovarian cancer (16,19,20). Following these studies, additional observational studies of NSAID use and ovarian cancer risk have been published.

We systematically searched the literature up to September 2012 and performed a meta-analysis of all observational studies of the associations between aspirin and non-aspirin NSAID use and ovarian cancer risk, including evaluations of dose/duration–response relationships and reasons for heterogeneity between studies. Most studies evaluated a combined outcome of invasive and borderline ovarian tumors. However, invasive and borderline ovarian tumors may be viewed as separate diseases, and therefore we performed additional analyses for invasive ovarian tumors only.

Material and methods

Literature search

Initially, we performed a literature search in the PubMed (US National Library of Medicine and the National Institute of Health) database. We used the following search terms: "analgesics" or "NSAIDs" or "aspirin" combined with "ovarian neoplasm*", "ovarian cancer" or "ovarian carcinoma*". Secondarily, we manually searched for additional studies by reviewing reference lists in retrieved articles and reviews. The titles and abstracts of all identified articles were screened to determine their relevance. Full articles were evaluated if the title and abstract were ambiguous. Duplicate publications were excluded.

Inclusion criteria and data extraction

Human studies, regardless of sample size, were included if they met the following inclusion criteria: (1) study published in English; (2) observational study (cohort study or case-control study); (3) study evaluating the association between aspirin and/or non-aspirin NSAIDs and risk of ovarian cancer; and (4) study reporting an estimate of association such as relative risk (RR), incidence rate ratio (IRR) or odds ratio (OR) with confidence intervals (CI). We made no restrictions on year of publication. When more than one study used data from the same study population, the most recent publication or the publication with the most complete information was included.

Data extraction was performed independently by two authors (L.B. and M.T.F.). Disagreements were resolved by consensus, referring back to the original article. We collected the following information from each article: author, year of publication, country of origin, study design, sample size, case definition, ascertainment of exposure, definition of regular use, confounder adjustment, lag time, and risk estimates with CI. When several estimates were presented, we chose the one adjusted for most variables.

Overall, the studies adjusted for a total of 34 different potential confounders. In Table 1, adjustment or matching for well-known risk factors (age, parity, oral contraceptive use, family history of ovarian and/or breast cancer, hormone replacement therapy use, tubal ligation, endometriosis/pelvic inflammatory disease, and smoking) are listed. Adjustment for additional potential risk factors is combined in the "others" category. One case-control study presented separate analyses using cancer and

Table 1. Characteristics and risk estimates for ever/never use of aspirin and non aspirin non-steroidal anti-inflammatory drugs (NSAIDs) in studies included in the meta-analysis.

Author (year) [ref.]	Study design	Study size Total (cases)	Case definition	Exposure ascertainment	Definition of regular use	Aspirin Estimate (95% CI)	Non-aspirin NSAIDs Estimate (95% CI)	Confounders[a]	Lag time (years)
Case-control studies									
Cramer (1998) USA (41)	Pop c/c	1086 (563)	Epithelial OC (incl. Borderline/LMP)	Interview	≥1 time/week for ≥6 months	0.78 (0.53–1.15)	1.20 (0.74–1.95)[b]	1–3,9	1
Rosenberg (2000) USA (21)	Hosp c/c	3350 (780)	Epithelial OC	Interview	≥4 days/week for ≥6 months	0.80 (0.50–1.20)	0.50 (0.30–0.90)	1,9	1
Tavani (2000) Italy (28)	Hosp c/c	1647 (749)	OC	Interview	≥1 time/week for >6 months	0.93 (0.53–1.62)	–	1–3,9	0
Akhmedkhanov (2001) USA (29)	Pop c/c	748 (68)	Epithelial OC (incl. Borderline/LMP)	Questionnaire	≥3 times/week for ≥6 months	0.60 (0.26–1.38)	–	1–4,9	1
Moysich (2001) USA (30)	Hosp c/c	1641 (547)	Epithelial OC	Questionnaire	≥1 time/week for ≥6 months	1.00 (0.73–1.39)	–	1,2,4,6,9	0
Meier (2002) UK (23)	Pop c/c	2360 (483)	OC	Database	>1 prescription	0.10 (0.02–1.00)	1.17 (0.95–1.43)[d]	1,8,9	1[e]
Schildkraut (2006) USA (31)	Pop c/c	1213 (586)	Epithelial OC (incl. Borderline/LMP)	Interview	≥3 months in 5 years prior to index	0.63 (0.39–1.02)	0.72 (0.43–1.21)[c]	1–4,6,7,9	0
Hannibal (2008) USA (32)	Pop c/c	2125 (812)	Epithelial OC (incl. Borderline/LMP)	Interview	≥5 days/month for ≥6 months	1.20 (0.90–1.50)	–	1–3,9	1
Merritt (2008) Australia (33)	Pop c/c	3085 (1576)	Epithelial OC (incl. Borderline/LMP)	Questionnaire	Any amount of use	1.06 (0.92–1.23)	0.88 (0.76–1.02)	1–3,9	1
Wernli (2008) USA (34)	Pop c/c	3140 (487)	Epithelial OC	Interview	≥2 times/week for ≥6 months	0.73 (0.54–1.00)	0.68 (0.50–0.93)[b]	1–4,6,9	0
Wu (2009) USA (22)	Pop c/c	1297 (609)	Epithelial OC (incl. Borderline/LMP)	Interview	≥2 times/week for ≥1 m	1.54 (1.10–2.15)[d]	1.81 (1.35–2.43)[d]	1–4,6,9	2
Lurie (2010) USA (35)	Pop c/c	2712 (1025)	OC	Interview	≥1 time/week for ≥6 months	0.84 (0.66–1.08)	0.79 (0.62–1.01)	1,3-5,9	0
Ammundsen (2012) Denmark (42)	Pop c/c	2320 (756)	Epithelial OC (incl. Borderline/LMP)	Interview	≥2 times/week for ≥1 month	0.68 (0.46–1.02)	1.06 (0.76–1.50)	1–3	1
Lo-Ciganic (2012) USA (36)	Pop c/c	2704 (902)	Epithelial OC (incl. Borderline/LMP)	Interview	≥2 tablets/week for ≥6 months	0.81 (0.63–1.03)	1.06 (0.83–1.36)	1–3,5,6,9	0
Cohort studies									
Friis (2003) Denmark (37)	Cohort	14 412 (34)	OC	Database	≥1 prescription	1.10 (0.70–1.50)	–	1	0
Sorensen (2003) Denmark (38)	Cohort	93 495 (130)	OC	Database	≥1 prescription	–	0.90 (0.70–1.00)	1	1
Lacey (2004) USA (39)	Cohort	31 364 (116)	Epithelial OC	Questionnaire	≥1 time/week for 1 year	0.86 (0.52–1.40)	1.00 (0.60–1.80)	1,3-5,9	1
Pinheiro (2009) USA (24)	Cohort	197 486 (666)	Epithelial OC (incl. Borderline/LMP)	Questionnaire	≥2 times/week	1.11 (0.92–1.33)	0.81 (0.64–1.01)	1-3,5,6,9	1

Table 1. Continued

Author (year) [ref.]	Study design	Study size Total (cases)	Case definition	Exposure ascertainment	Definition of regular use	Aspirin Estimate (95% CI)	Non-aspirin NSAIDs Estimate (95% CI)	Confounders[a]	Lag time (years)
Prizment (2010) USA (40)	Cohort	21 694 (157)	Epithelial OC	Questionnaire	Any amount of use	0.77 (0.54–1.10)	0.89 (0.64–1.23)	1,2,5,9	2/3[e]
Murphy (2012) USA (20)	Cohort	96 710 (438)	Epithelial OC	Questionnaire	≥ 1 tablet/week	1.06 (0.87–1.29)	0.93 (0.74–1.15)	1,2,3,4,5,9	1/2[e]
Setiawan (2012) USA (43)	Cohort	64 387 (275)	Epithelial OC	Questionnaire	≥ 2 times/week for ≥ 1 month	0.87 (0.68–1.14)	0.97 (0.74–1.26)	1,2,3,5,9	2[e]

[a]Confounders: 1, age; 2, parity; 3, oral contraceptive use; 4, family history of ovarian and/or breast cancer; 5, hormone replacement therapy use; 6, tubal ligation; 7, endometriosis and/or pelvic inflammatory disease; 8, smoking; 9,others;
[b]Estimate based on use of ibuprofen;
[c]Estimate based on use of COX-2 inhibitors;
[d]Crude risk estimate;
[e]Lag time analyses were conducted but presented results are without lag time.
Abbreviations: Hosp *c/c*, hospital case-control study; LMP, low malignant potential; OC, ovarian cancer; Pop *c/c*, population case-control study.

non-cancer control groups, respectively (21). In the present meta-analysis, we included estimates derived from the analysis using non-cancer controls.

We focused on use of aspirin and non-aspirin NSAIDs, separately. Therefore, estimates on use of aspirin and non-aspirin NSAIDs combined were not included in the meta-analysis.

Definitions of regular use varied considerably between studies. From each study, we extracted the estimate representing ever/never regular use of aspirin or non-aspirin NSAID. Two case-control studies presented no ever/never estimates (22,23); however, based on the available data, we were able to calculate crude ever/never ORs for these two studies for the purpose of our meta-analysis. One cohort study reported an ever/never risk estimate on current and past use (24). As any chemopreventive effect of NSAIDs would be likely to decline gradually after discontinuation, we chose to include the risk estimate for current use in the meta-analysis. We also extracted the highest intake presented in each study, either the highest dose or the longest duration of use. In studies reporting on both frequency and duration of use, we included estimates on long-term use.

Statistical analyses

We computed pooled RRs with corresponding 95% CI for each type of drug, i.e. aspirin and non-aspirin NSAIDs, using both fixed and random effect models. The fixed effect model assumes that the underlying true exposure effect in the pooled studies is the same (25). Random effect models take into account variation between studies, i.e. that each study has its own (true) exposure effect (25). We chose to present random effect pooled RR. The assumption of variation is plausible given the differences in study design, ascertainment of exposure, definition of regular use of analgesic use, etc. The pooled estimates on ever/never use of aspirin and non-aspirin NSAIDs are presented graphically using forest plots (Figures 1 and 2). Results from the remaining meta-analyses are presented in table format (Table 2).

The extent of statistical heterogeneity was evaluated using the quantity I^2 (the between-studies variance divided by the within-study variance plus the between-studies variance times 100). I^2 describes the percentage of total variation that is due to heterogeneity rather than chance. A value of 0% indicates no observed heterogeneity, whereas values of 25, 50 and 75% are equal to low, moderate and high levels of heterogeneity (26). Furthermore, we aimed to analyze the origin of heterogeneity in sub-analyses according to study design (case-control vs. cohort study, and population-based vs. hospital-based case-control study) and case definition (invasive ovarian

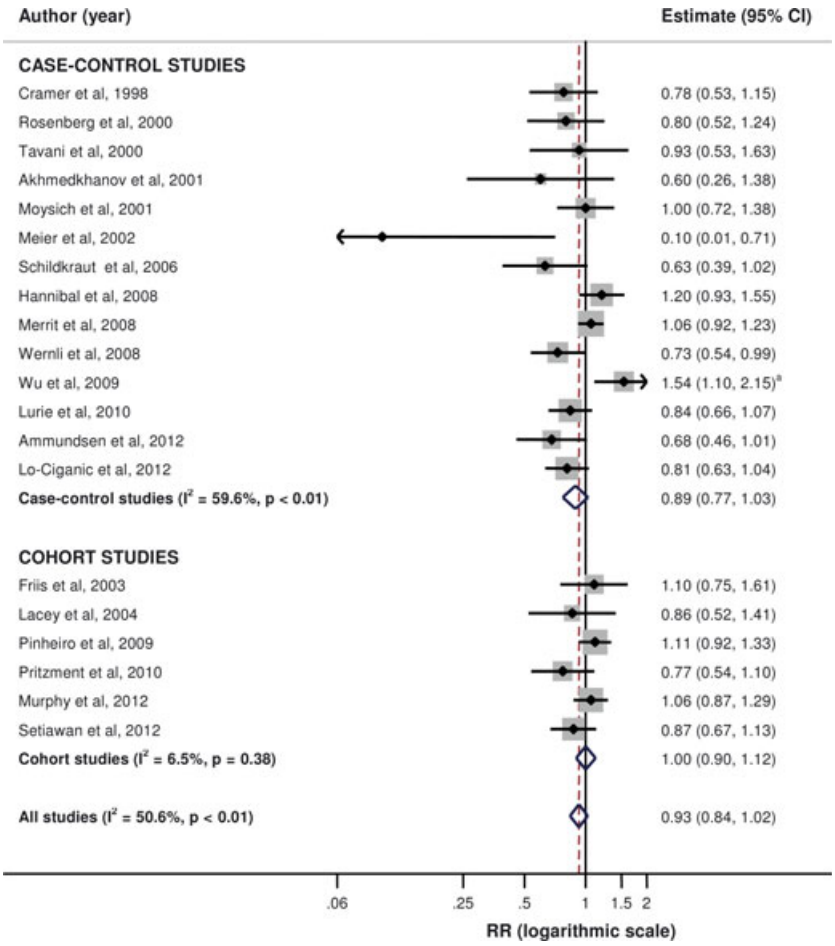

Figure 1. Forest plot of the association between aspirin use and risk of ovarian cancer. Pooled estimates are based on a random effect model. Squares and lines indicate the study-specific risk estimate and confidence interval. The size of the square is proportional to the study weight calculated as the inverse of the within-study variance plus the between-studies variance. The overall estimate of the analysis is given by a diamond; the center represents the pooled relative risk (RR) and the ends represent the 95% confidence interval (CI). [a]Crude risk estimate.

cancer with or without borderline tumors). To evaluate dose/duration-response relationships, we also performed a meta-analysis on maximum use as defined in each study.

Potential publication bias was evaluated graphically with funnel plots of risk estimates against the standard error of the study. In the plots, the vertical line is drawn at the pooled RR. The funnel plots were supplemented with formal testing using linear regression as proposed by Egger et al. (27). Using this test, funnel plot asymmetry is measured by determining whether the intercept deviates statistically significantly from zero. Analyses were performed using STATA Statistical Software version 11 (StataCorp., College Station, TX, USA).

Results

Search results

We identified 742 potentially relevant articles in PubMed until 17 September 2012. Based on the title and abstract review, we excluded 80 articles that were not in English, 60 review articles, 326 preclinical reports (i.e. animal studies or *in vitro* cell line experiments), 130 clinical experimental reports, and 122 articles that did not report the main subject of interest. After exclusion, 24 relevant articles remained. By manually reviewing reference lists in these, we added five more articles. Of the 29 identified studies, 21 (20–24,28–43) met the inclusion criteria for full article review. The remaining eight studies were excluded because outcome was death and not risk of ovarian cancer (n = 1) (44), information on specific types of analgesics was missing (n = 1) (45), risk estimates associated with cancer types other than ovarian cancer were reported (n = 3) (46–48), or because data from the same study was presented in one of the other included studies (n = 3) (49–51). This yielded a total of 21 studies for the meta-analysis.

Study characteristics

Characteristics of the included studies are shown in Table 1. Fourteen studies were case-control studies (21–23,28–36,41,42) and seven were cohort studies (20,24,37–40,43). The majority of the case-control studies were population-based (n = 11) (22,23,29,31–36,41,42),

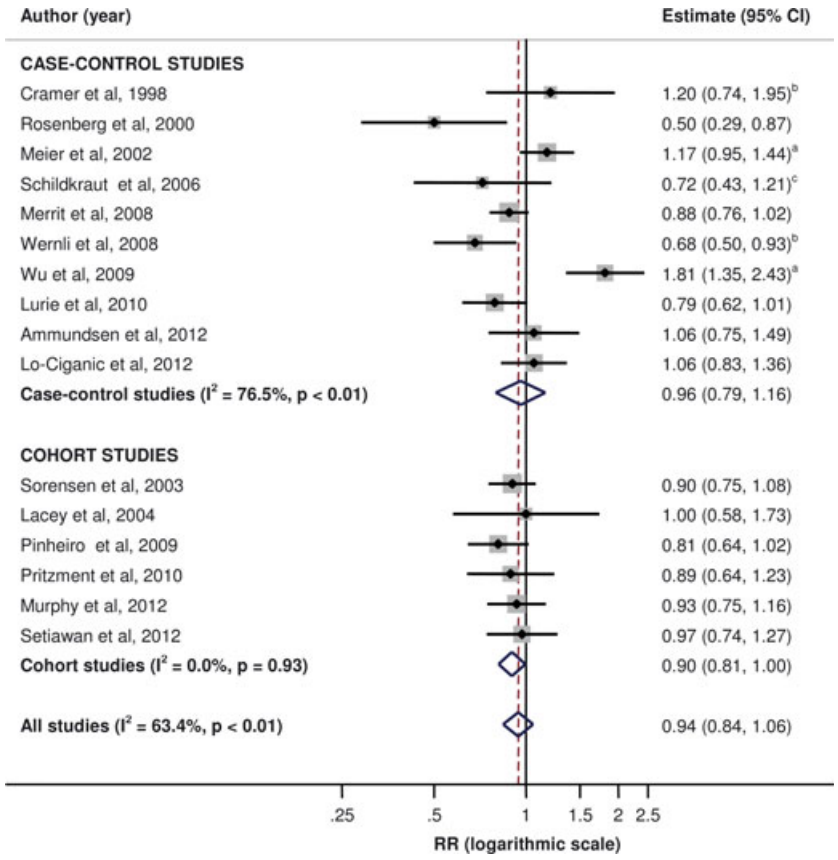

Figure 2. Forest plot of the association between use of non-aspirin NSAIDs and risk of ovarian cancer. Pooled estimates are based on a random effect model. Squares and lines indicate the study-specific risk estimate and confidence interval. The size of the square is proportional to the study weight calculated as the inverse of the within-study variance plus the between-studies variance. The overall estimate of the analysis is given by a diamond; the center represents the pooled relative risk (RR) and the ends represent the 95% confidence interval (CI). [a]Crude risk estimate. [b]Estimate based on use of ibuprofen. [c]Estimate based on use of COX-2 inhibitors.

whereas the remaining three case-control studies were hospital-based (21,28,30). In most studies, cases were women with ovarian cancer of epithelial origin (20–22,24,29–34,36,39–43). Eight studies reported estimates based on invasive and borderline ovarian tumors combined (22,24,29,31–33,41,42) and 12 studies investigated the risk for invasive ovarian cancer only (20,21,23,28,30,34,35,37–40,43). One study examined both invasive and borderline tumors combined and invasive ovarian cancer separately (36). Apart from one small case-control study, including 68 cases (29), the remaining case-control studies were moderate to large study populations including between 483 and 1576 cases. In the cohort studies, the number of cases ranged from 34 to 666. Most studies were conducted in the USA (n = 15) (20–22,24,29–32,34–36,39–41,43), one study was from Australia (33), and the remaining five studies originated from Europe (23,28,37,38,42). Twenty studies measured exposure to aspirin (20–24,28–37,39–43), whereas 16 studies reported risk estimates for use of non-aspirin NSAIDs (20–24,31,33–36,38–43). Regarding exposure assessment, the majority of the studies used interviews (21,22,28,31,32,34–36,41,42) or self-administered questionnaires (20,24,29,30,33,39,40,43). In the remaining studies, the information was retrieved from databases (23,37,38). Definition of regular use of aspirin and non-aspirin NSAIDs varied substantially between studies. In some studies, regular use was defined by consumption of a specific amount of NSAID for a minimum of one month (22,42,43), six months (21,28–30,32,34–36,41) or one year (39). Other studies used a broader definition including any amount of use (33,40), redemption of more than one prescription (23) or one or more prescriptions (37,38). More than half of the studies took lag time into consideration, i.e. disregarded NSAID exposure in a defined period before diagnosis (20–24,29,32,33,38–43). All studies either matched or adjusted for age. Among the well-known risk factors, most studies adjusted for parity (20,22,24,28–34,36,40–43), oral contraceptive use (20,22,24,28,29,31–36,39,41–43) and family history of ovarian and/or breast cancer (20,22,29–31,34,35,39).

Meta-analysis on the effect of aspirin

Figure 1 presents individual and summary risk estimates for the 18 studies evaluating the association between use (ever vs. never) of aspirin and risk of ovarian cancer. In the overall analysis combining case-control and cohort studies, use of aspirin was associated with a slightly, although not statistically significant, decreased

Table 2. Pooled risk estimates in stratified analyses according to use of aspirin and non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs).

Analysis	No. of studies	RR (95% CI)	Heterogeneity, I^2	*p* for I^2
Aspirin				
Maximum use, overall (c-c and cohort) (21,22,24,28–33,36,39,40,42,43)	14	0.89 (0.74–1.06)	55.0%	<0.01
Maximum use, c-c studies (21,22,28–33,36,42)	10	0.93 (0.72–1.19)	62.8%	<0.01
Maximum use, cohort studies (24,39,40,43)	4	0.82 (0.64–1.05)	25.2%	0.26
Ever use, pop c-c (22,23,29,31–36,41,42)	11	0.88 (0.74–1.05)	68.3%	<0.01
Ever use, hosp c-c (21,28,30)	3	0.93 (0.73–1.17)	0.0%	0.72
Ever use, invasive ovarian cancer only (20,21,23,28,30,34–37,39,40,43)[a]	12	0.88 (0.79–0.98)	16.2%	0.29
Ever use, invasive ovarian cancer and borderline ovarian tumors (22,24,29,31–33,36,41,42)[a]	9	0.96 (0.81–1.13)	65.3%	<0.01
Non-aspirin NSAIDs				
Maximum use, overall (c-c and cohort) (21–24,31,33,36,38–40,42,43)	12	0.99 (0.84–1.17)	32.2%	0.13
Maximum use, c-c studies (21–23,31,33,36,42)	7	1.02 (0.81–1.29)	39.0%	0.13
Maximum use, cohort studies (24,38–40,43)	5	0.97 (0.73–1.27)	37.2%	0.17
Ever use, pop c-c (22,23,31,33–36,41,42)	9	1.00 (0.83–1.20)	75.5%	<0.01
Ever use, hosp c-c (21)	1	0.50 (0.29–0.87)	–	–
Ever use, invasive ovarian cancer only (20,21,23,34–36,38–40,43)[a]	10	0.91 (0.81–1.02)	45.9%	0.06
Ever use, invasive ovarian cancer and borderline ovarian tumors (22,24,31,33,36,41,42)[a]	7	1.03 (0.84–1.28)	75.1%	<0.01

aThe study by Lo Ciganic et al. (36) provided data for both the analysis on invasive ovarian cancer only and the analysis where borderline ovarian tumors were included among cases.

CI, confidence interval; I^2,the between-studies variance divided by the within-study variance plus the between-studies variance times 100; Hosp c/c, hospital case-control study; Pop c/c, population case-control study; RR, relative risk.

risk of ovarian cancer (RR 0.93; 95% CI 0.84–1.02). Heterogeneity between studies was substantial (I^2 = 50.6%; $p < 0.01$). After stratification by study design, the pooled risk estimate among case-control studies was virtually unchanged (RR 0.89; 95% CI 0.77–1.03) and heterogeneity remained high (I^2 = 59.6%; $p < 0.01$). Among cohort studies, the risk estimate was at unity and heterogeneity was low (RR 1.00; 95% CI 0.90–1.12; I^2 = 6.5%; p = 0.38).

Pooled estimates of sub-analyses are presented in Table 2. For high intake of aspirin, the pooled risk estimate in the overall analysis was similar to the corresponding estimate associated with ever use of aspirin (RR 0.89; 95% CI 0.74–1.06; I^2 = 55.0%; $p < 0.01$). The same applied to the analysis including only case-control studies, whereas the risk estimate for cohort studies was reduced compared with the overall estimate (RR 0.82; 95% CI 0.64–1.05; I^2 = 25.2%; p = 0.26) (Table 2).

Stratification of case-control studies according to the underlying study base, i.e. population-based or hospital-based studies, yielded similar risk estimates. Only three studies were hospital-based and heterogeneity between those was eliminated. Among the population-based studies, heterogeneity remained high (I^2 = 68.3%; $p < 0.01$). Finally, studies were divided according to presence of borderline tumors among ovarian cancer cases. For invasive ovarian cancer only, use of aspirin was associated with a statistically significantly reduced RR of 0.88 (95% CI 0.79–0.98) and heterogeneity was low (I^2 = 16.2%; p = 0.29) (Table 2).

Meta-analysis on the effect of non-aspirin NSAIDs

The meta-analysis of the ten case-control studies and the six cohort studies with results on non-aspirin NSAID indicated an inverse association between ever vs. never use of non-aspirin NSAIDs and risk of ovarian cancer (RR 0.94; 95% CI 0.84–1.06, Figure 2), although it was not statistically significant. Heterogeneity was high (I^2 = 63.4%; $p < 0.01$). When we analyzed case-control studies separately, the pooled estimate and the level of heterogeneity were virtually similar to the overall analysis, whereas among cohort studies only, we found a statistically significantly protective effect of non-aspirin NSAIDs (RR 0.90; 95% CI 0.81–1.00, p = 0.04) with no important heterogeneity.

Twelve studies were eligible for analyses on maximum use of non-aspirin NSAIDs and type of study design (Table 2). We found no evidence of an increased inverse association in these analyses. Only one case-control study was hospital-based. Excluding this study yielded virtually similar results as the analysis including all case-control studies (RR 1.00; 95% CI 0.83–1.20; I^2 = 75.5%; $p < 0.01$). When including only studies of invasive ovarian cancer, the pooled RR was 0.91 (95% CI 0.81–1.02) with moderate heterogeneity between studies (I^2 = 45.9%; p = 0.06) (Table 2).

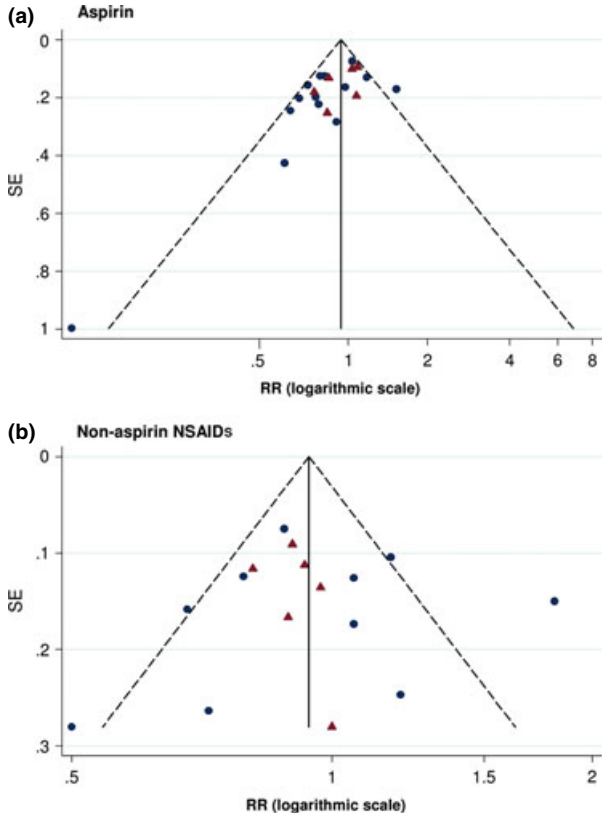

Figure 3. Funnel plots of the standard error (SE) of relative risk (RR) for ovarian cancer associated with use of aspirin (a) and non-aspirin NSAIDs (b). Circles indicate case-control studies and triangles indicate cohort studies. Note that the scale of the X and Y-axis are different in Figure 3a and 3b: In Figure 3a, Egger's test *p*-value = 0.01; in Figure 3b, Egger's test *p*-value = 0.91.

Publication bias

Graphical funnel plots of the standard error of the RR for ovarian cancer for the studies in the present meta-analysis are presented in Figure 3a and b. Using the Egger regression test, we found evidence of publication bias in the meta-analysis of the association between aspirin and ovarian cancer ($p = 0.01$). Regarding studies reporting on the association between non-aspirin NSAIDs and ovarian cancer, the *p*-value for the Egger regression test was 0.91, indicating that publication bias was not present.

Discussion

In our meta-analysis, we found a statistically significantly decreased risk of invasive ovarian cancer associated with aspirin use with no important heterogeneity between studies. Furthermore, our results indicated an inverse association between use of non-aspirin NSAIDs and risk of invasive ovarian cancer, but the risk estimate was statistically non-significant. In the analyses combining invasive and borderline ovarian tumors, we found a tendency towards an inverse association between use of aspirin and non-aspirin NSAIDs and ovarian cancer risk. Focusing on maximum use of aspirin and non-aspirin NSAIDs, no dose-response effect was found. In general, our analyses revealed high between-study heterogeneity, especially among case-control studies.

Our results are consistent with previous meta-analyses (15–19). In one recent analysis by Ni et al. (19), the authors reported pooled (random effect model) risk estimates on use of aspirin and non-aspirin NSAIDs of 0.91 (95% CI: 0.82–1.01) and 0.89 (95% CI: 0.74–1.08), respectively. Compatible with our findings, no apparent association was observed between NSAID dose and risk of ovarian cancer (19). In another recent meta-analysis, including 13 studies, Murphy et al. (20) found no evidence of an association between aspirin use and ovarian cancer risk, but a slight inverse association between use of non-aspirin NSAIDs and ovarian cancer.

Despite the high statistical precision in our study, the high between-study heterogeneity precludes firm conclusions on the associations. In our study, we additionally addressed associations according to the malignancy of the ovarian tumors and found indications that a potential chemopreventive effect of NSAIDs against ovarian cancer may be restricted to invasive ovarian cancer, particularly for aspirin. Eight studies included both invasive ovarian cancer and borderline ovarian tumors in the case group without reporting separate estimates for invasive tumors (22,24,29,31–33,41,42). Although most of these studies indicated that exclusion of borderline tumors did not affect the results (24,29,31,32,42), our findings emphasize that separate analyses are justified.

The between-study heterogeneity was highest among case-control studies. One important source of heterogeneity between the studies was the large variation in the definition of use. Exposure varied from any use (33,40) to four days or more per week for at least six months (21). With such variation, exposed women and controls are not comparable across studies. In the analysis on maximum intake of NSAIDs, we gave precedence to long-term use over high frequency of use. This is in accordance with a recent secondary analysis from randomized clinical trials of aspirin reporting that the chemopreventive effect of aspirin is most evident after more than five years treatment (52). Although most studies of NSAIDs and ovarian cancer reported an estimate on use for more than five or 10 years, the exposure definition in these periods remained diverse.

Difference in the methods of exposure assessment was another source of between-study heterogeneity. Studies using self-administered questionnaires or interviews are

susceptible to recall bias. Three studies were based on prescription databases (23,37,38) containing detailed information on specific type of drug, number of prescriptions and time of use. However, these studies are limited by the lack of information on NSAIDs obtained over-the-counter (OTC), which many claim is a serious limitation of studies of NSAIDs. Using the United Kingdom-based General Practice Research Database, Meier et al. (23) explored the extent to which women registered as unexposed in the database used NSAIDs OTC. Although that study was based on a limited number of women, the analysis provided evidence that considerable exposure misclassification was rare (23).

Selection bias may also influence the study results, especially in case-control studies. In hospital-based case-control studies, the controls might have a higher frequency of NSAID use than the general population. In our meta-analysis of aspirin, we did not find any major difference in the pooled risk estimates when we stratified the analysis according to population-based and hospital-based case-control studies. Heterogeneity remained high among population-based case-control studies, whereas heterogeneity was largely eliminated among the hospital-based studies. In the latter analysis, however, only three hospital-based studies were included and the test for heterogeneity loses sensitivity when only a small number of studies are pooled. In the analysis on non-aspirin NSAIDs, only one study was hospital-based.

Confounding should also be considered as a potential major contributor to the variance between studies. Two studies only adjusted for age (37,38), but the majority of the studies adjusted for most well-established risk factors for ovarian cancer such as parity, oral contraceptive use, and family history of ovarian and/or breast cancer. Therefore, variation in adjustment for confounders is probably not a major source of the observed heterogeneity in the present study. Finally, only a few studies incorporated a lag time period in the exposure definition. Use of analgesics close to the diagnosis of ovarian cancer could be associated with early manifestations of the disease giving rise to "protopathic" bias (reverse causation) (53,54). Thus, a chemopreventive effect of NSAIDs might be underestimated in studies not including a lag time due to a disease-related higher prevalence of analgesic use among cases before diagnosis.

In contrast to the inconclusive results of observational studies of the association between NSAIDs and ovarian cancer risk, several preclinical studies have demonstrated an inhibitory effect of NSAIDs against ovarian neoplasia and suggested possible biological mechanisms. A chemopreventive effect of NSAIDs may occur through inhibition of the COX-enzymes involved in prostaglandin synthesis and inflammation (5). It has been demonstrated that COX-enzymes are up-regulated in ovarian cancer cells (13,55). Elevated levels of COX might promote tumor progression and stimulate tumor angiogenesis (55). Furthermore, the COX enzymatic activity might interact with cellular signaling pathways resulting in activation of proteins with oncogenic and anti-apoptotic properties (13). In addition to the findings from in vitro and in vivo studies, the increasing evidence of a general association between chronic inflammation and ovarian cancer (10) also supports the hypothesis of a chemopreventive effect of NSAIDs against ovarian cancer.

Some limitations of the present meta-analysis should be mentioned. First, the literature search was restricted to published studies and we only included studies in English. These limitations might explain why we found evidence of publication bias in the analysis of aspirin use and ovarian cancer. However, no apparent publication bias was found in the analysis of non-aspirin NSAIDs. Secondly, all included studies were observational. We made this restriction because almost all studies examining the association between NSAIDs and ovarian cancer are either case-control or cohort studies. Compared with randomized controlled trials, however, observational studies are more susceptible to bias and confounding. We tried to reduce confounding by extracting the estimate in each study which was adjusted for most variables. Thirdly, due to the large variation in the exposure definitions, we were only able to evaluate the effect of maximum use and not whether there was a potential dose/duration-response effect. Finally, we found substantial between-studies variance. Although the random effect model takes heterogeneity into account, this is a limitation and our results should be interpreted with caution.

Based on this meta-analysis, the association between aspirin and non-aspirin NSAID use and ovarian cancer risk is weak. However, stratified analysis indicated that a chemopreventive effect might be restricted to invasive ovarian tumors, which emphasizes the importance of analyzing invasive and borderline ovarian tumors separately in future studies. We found substantial heterogeneity between studies and therefore results should be interpreted cautiously. The hypothesis of a protective role of NSAIDs against ovarian cancer is supported by preclinical studies and the indications of a causal association between chronic inflammation and ovarian cancer. Further robust and well-designed population-based studies of the association between NSAIDs and ovarian cancer risk are warranted.

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References

1. Kjaerbye-Thygesen A, Huusom LD, Frederiksen K, Kjaer SK. Trends in the incidence and mortality of ovarian cancer in Denmark 1978–2002. Comparison with other Nordic countries. Acta Obstet Gynecol Scand. 2005;84:1006–12.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127:2893–917.
3. GLOBOCAN. Available from: http://globocan.iarc.fr (accessed 27 January 2012).
4. Hannibal CG, Cortes R, Engholm G, Kjaer SK. Survival of ovarian cancer patients in Denmark: excess mortality risk analysis of five-year relative survival in the period 1978–2002. Acta Obstet Gynecol Scand. 2008;87:1353–60.
5. Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer. 2006;6:130–40.
6. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer. 2007;121:2373–80.
7. Mills GB. Mechanisms underlying chemoprevention of ovarian cancer. Clin Cancer Res. 2002;8:7–10.
8. Elwood PC, Gallagher AM, Duthie GG, Mur LA, Morgan G. Aspirin, salicylates, and cancer. Lancet. 2009;373:1301–9.
9. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol. 2012;13:518–27.
10. Ness RB, Cottreau C. Possible role of ovarian epithelial inflammation in ovarian cancer. J Natl Cancer Inst. 1999;91:1459–67.
11. Shan W, Liu J. Inflammation: a hidden path to breaking the spell of ovarian cancer. Cell Cycle. 2009;8:3107–11.
12. Ness RB, Grisso JA, Cottreau C, Klapper J, Vergona R, Wheeler JE, et al. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. Epidemiology. 2000;11:111–17.
13. Uddin S, Ahmed M, Hussain A, Assad L, Al-Dayel F, Bavi P, et al. Cyclooxygenase-2 inhibition inhibits PI3K/AKT kinase activity in epithelial ovarian cancer. Int J Cancer. 2010;126:382–94.
14. Rodriguez-Burford C, Barnes MN, Oelschlager DK, Myers RB, Talley LI, Partridge EE, et al. Effects of nonsteroidal anti-inflammatory agents (NSAIDs) on ovarian carcinoma cell lines: preclinical evaluation of NSAIDs as chemopreventive agents. Clin Cancer Res. 2002;8:202–9.
15. Gonzalez-Perez A, Garcia Rodriguez LA, Lopez-Ridaura R. Effects of non-steroidal anti-inflammatory drugs on cancer sites other than the colon and rectum: a meta-analysis. BMC Cancer. 2003;3:28.
16. Bonovas S, Filioussi K, Sitaras NM. Do nonsteroidal anti-inflammatory drugs affect the risk of developing ovarian cancer? A meta-analysis. Br J Clin Pharmacol. 2005;60:194–203.
17. Bosetti C, Gallus S, La VC. Aspirin and cancer risk: an updated quantitative review to 2005. Cancer Causes Control. 2006;17:871–88.
18. Cuzick J, Otto F, Baron JA, Brown PH, Burn J, Greenwald P, et al. Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. Lancet Oncol. 2009;10:501–7.
19. Ni X, Ma J, Zhao Y, Wang Y, Wang S. Meta-analysis on the association between non-steroidal anti-inflammatory drug use and ovarian cancer. Br J Clin Pharmacol. 2012;75:26–35.
20. Murphy MA, Trabert B, Yang HP, Park Y, Brinton LA, Hartge P, et al. Non-steroidal anti-inflammatory drug use and ovarian cancer risk: findings from the NIH-AARP Diet and Health Study and systematic review. Cancer Causes Control. 2012;23:1839–52.
21. Rosenberg L, Palmer JR, Rao RS, Coogan PF, Strom BL, Zauber AG, et al. A case-control study of analgesic use and ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2000;9:933–7.
22. Wu AH, Pearce CL, Tseng CC, Templeman C, Pike MC. Markers of inflammation and risk of ovarian cancer in Los Angeles County. Int J Cancer. 2009;124:1409–15.
23. Meier CR, Schmitz S, Jick H. Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer. Pharmacotherapy. 2002;22:303–9.
24. Pinheiro SP, Tworoger SS, Cramer DW, Rosner BA, Hankinson SE. Use of nonsteroidal antiinflammatory agents and incidence of ovarian cancer in 2 large prospective cohorts. Am J Epidemiol. 2009;169:1378–87.
25. Blettner M, Sauerbrei W, Schlehofer B, Scheuchenpflug T, Friedenreich C. Traditional reviews, meta-analyses and pooled analyses in epidemiology. Int J Epidemiol. 1999;28:1–9.
26. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Br Med J. 2003;327:557–60.
27. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. Br Med J. 1997;315:629–34.
28. Tavani A, Gallus S, La Vecchia C, Conti E, Montella M, Franceschi S. Aspirin and ovarian cancer: an Italian case-control study. Ann Oncol. 2000;11:1171–3.
29. Akhmedkhanov A, Toniolo P, Zeleniuch-Jacquotte A, Kato I, Koenig KL, Shore RE. Aspirin and epithelial ovarian cancer. Prev Med. 2001;33:682–7.
30. Moysich KB, Mettlin C, Piver MS, Natarajan N, Menezes RJ, Swede H. Regular use of analgesic drugs and ovarian cancer risk. Cancer Epidemiol Biomarkers Prev. 2001;10:903–6.

31. Schildkraut JM, Moorman PG, Halabi S, Calingaert B, Marks JR, Berchuck A. Analgesic drug use and risk of ovarian cancer. Epidemiology. 2006;17:104–7.
32. Hannibal CG, Rossing MA, Wicklund KG, Cushing-Haugen KL. Analgesic drug use and risk of epithelial ovarian cancer. Am J Epidemiol. 2008;167:1430–7.
33. Merritt MA, Green AC, Nagle CM, Webb PM. Talcum powder, chronic pelvic inflammation and NSAIDs in relation to risk of epithelial ovarian cancer. Int J Cancer. 2008;122:170–6.
34. Wernli KJ, Newcomb PA, Hampton JM, Trentham-Dietz A, Egan KM. Inverse association of NSAID use and ovarian cancer in relation to oral contraceptive use and parity. Br J Cancer. 2008;98:1781–3.
35. Lurie G, Terry KL, Wilkens LR, Thompson PJ, McDuffie KE, Carney ME, et al. Pooled analysis of the association of PTGS2 rs5275 polymorphism and NSAID use with invasive ovarian carcinoma risk. Cancer Causes Control. 2010;21:1731–41.
36. Lo-Ciganic WH, Zgibor JC, Bunker CH, Moysich KB, Edwards RP, Ness RB. Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, or acetaminophen and risk of ovarian cancer. Epidemiology. 2012;23:311–9.
37. Friis S, Sorensen HT, McLaughlin JK, Johnsen SP, Blot WJ, Olsen JH. A population-based cohort study of the risk of colorectal and other cancers among users of low-dose aspirin. Br J Cancer. 2003;88:684–8.
38. Sørensen HT, Friis S, Nørgard B, Mellemkjær L, Blot WJ, McLaughlin JK, et al. Risk of cancer in a large cohort of nonaspirin NSAID users: a population-based study. Br J Cancer. 2003;88:1687–92.
39. Lacey JV Jr, Sherman ME, Hartge P, Schatzkin A, Schairer C. Medication use and risk of ovarian carcinoma: a prospective study. Int J Cancer. 2004;108:281–6.
40. Prizment AE, Folsom AR, Anderson KE. Nonsteroidal anti-inflammatory drugs and risk for ovarian and endometrial cancers in the Iowa Women's Health Study. Cancer Epidemiol Biomarkers Prev. 2010;19:435–42.
41. Cramer DW, Harlow BL, Titus-Ernstoff L, Bohlke K, Welch WR, Greenberg ER. Over-the-counter analgesics and risk of ovarian cancer. Lancet. 1998;351:104–7.
42. Ammundsen HB, Faber MT, Jensen A, Høgdall E, Blaakær J, Høgdall C, et al. Use of analgesic drugs and risk of ovarian cancer – Results from a Danish case-control study. Acta Obstet Gynecol Scand. 2012;91:1094–1102.
43. Setiawan VW, Matsuno RK, Lurie G, Wilkens LR, Carney ME, Henderson BE, et al. Use of nonsteroidal anti-inflammatory drugs and risk of ovarian and endometrial cancer: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev. 2012;21:1441–9.
44. Rodriguez C, Henley SJ, Calle EE, Thun MJ. Paracetamol and risk of ovarian cancer mortality in a prospective study of women in the USA. Lancet. 1998;352:1354–5.
45. Tzonou A, Polychronopoulou A, Hsieh CC, Rebelakos A, Karakatsani A, Trichopoulos D. Hair dyes, analgesics, tranquilizers and perineal talc application as risk factors for ovarian cancer. Int J Cancer. 1993;55:408–10.
46. Langman MJ, Cheng KK, Gilman EA, Lancashire RJ. Effect of anti-inflammatory drugs on overall risk of common cancer: case-control study in general practice research database. Br Med J. 2000;320:1642–6.
47. Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW Jr. Aspirin use and risk of fatal cancer. Cancer Res. 1993;53:1322–7.
48. Friedman GD, Ury HK. Initial screening for carcinogenicity of commonly used drugs. J Natl Cancer Inst. 1980;65:723–33.
49. Pinheiro SP, Gates MA, Devivo I, Rosner BA, Tworoger SS, Titus-Ernstoff L, et al. Interaction between use of non-steroidal anti-inflammatory drugs and selected genetic polymorphisms in ovarian cancer risk. Int J Mol Epidemiol Genet. 2010;1:320–31.
50. Fairfield KM, Hunter DJ, Fuchs CS, Colditz GA, Hankinson SE. Aspirin, other NSAIDs, and ovarian cancer risk (United States). Cancer Causes Control. 2002;13:535–42.
51. Rosenberg L. Nonsteroidal anti-inflammatory drugs and cancer. Prev Med. 1995;24:107–9.
52. Rothwell PM, Price JF, Fowkes FGR, Zanchetti A, Roncaglioni MC, Tognoni G, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet. 2012;379:1602–12.
53. Horwitz RI, Feinstein AR. The problem of "protopathic bias" in case-control studies. Am J Med. 1980;68:255–8.
54. Csizmadl I, Collet J-P, Boivin J. Bias and confounding in pharmacoepidemiology. In: Strom BL (ed.). *Pharmacoepidemiology*, 4th edn. Bognor Regis: Wiley and Sons, 2007. pp. 791–810.
55. Lee JS, Choi YD, Lee JH, Nam JH, Choi C, Lee MC, et al. Expression of cyclooxygenase-2 in epithelial ovarian tumors and its relation to vascular endothelial growth factor and p53 expression. Int J Gynecol Cancer. 2006;16 (Suppl 1):247–53.